Advances in critical fluid technology for food processing

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The use of supercritical or near critical fluids for food processing has an established thirty year history in the industry. However in the last five years, substantial advances in how critical fluid processing is conducted have been developed; in our laboratory and throughout the world. This article highlights some of the new possibilities for employing these novel processing media, not only for extraction of foodstuffs, but for enrichment or fractionation of key components (i.e., nutraceuticals), as well as options for conducting reactions in critical fluids. A particular focus is laid on enrichment methodologies, such as fractionation towers and preparative chromatography, for enhancing active ingredients in raw material sources. Alternative media are also mentioned, such as subcritical water or environmentally-compatible fluorocarbons which have no negative impact on the resultant products when used in a complimentary role to supercritical carbon dioxide.

Introduction

The use of supercritical fluids as processing agents in the food industry has resulted in several processes and products and the construction of over thirty processing plants of varying scale, world-wide during the past 30 years. Well known processes such as the decaffeination of coffee, or the fractionation of hops for the flavoring of beer, have been abetted by more recent processes producing an array of food-related products. These products have included spice and flavor extracts (1), defatted or reduced-cholesterol products (2), natural antioxidants (3), and specialized oil-derived products for the nutritional market (4).

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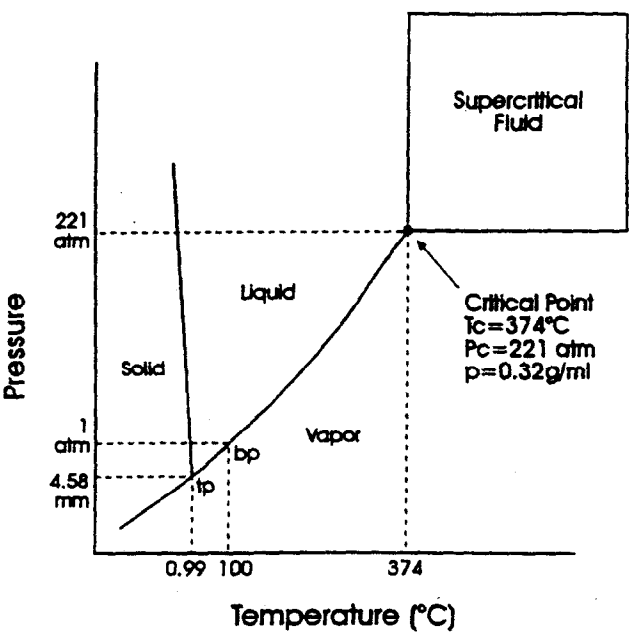

Figure. 1 - Phase diagram for water as a function of temperature and pressure.

Today we use the term 'critical fluid' to embrace a variety of processing methods having in common the application of pressure to achieve a desired end result. For example, if we examine the phase diagram for water (Figure 1), we can see that the critical point is defined by a specific temperature and pressure, in this case, 374°C and 221 atmospheres, respectively. Therefore, trying to conduct extractions in the supercritical fluid regime would obviously be deleterious to many food-related components, however this does not mean that water cannot be used as a processing agent under pressure for extraction, etc. purposes.

Note that below the critical temperature there exists a wide region, where water if compressed, will retain its liquid state. This is frequently called the subcritical state of water. This near critical region in the water phase diagram is analogous to the near critical state of CO_2 which is sometimes referred to as liquified CO_2. Hence, by carefully controlling the extraction conditions one has a 'natural' medium for extractions, etc (5). The reason why the phase diagram of water was chosen is largely for illustrative purposes and to shake the reader from his/her lethargy, since one might expect to see a phase diagram for carbon dioxide, by far the most popular supercritical or near critical fluid. However it also justifies the use of the term 'critical fluid' technology in a generic sense, to embody fluids and conditions that have applicability for processing foods aside from SC-CO_2.

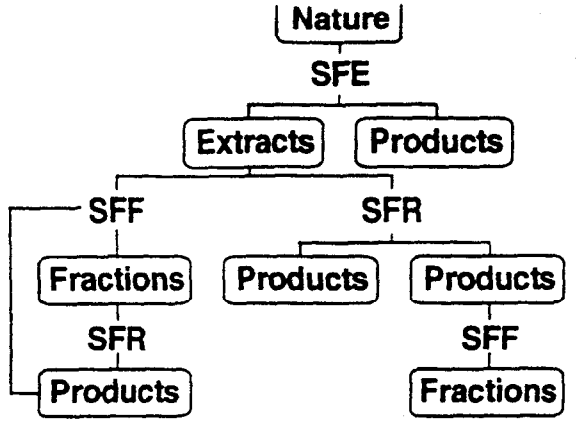

Figure 2 - Processing sequences utilizing critical fluid media for isolating or synthesizing end products.

In addition it is important to note that the nature of critical fluid processing has also changed in the last 30 years. No longer is supercritical fluid extraction (SFE) the only method used for the isolation of specific components from natural matrices. As shown in Figure 2, fractionation and reaction modes utilizing supercritical fluids are becoming more common, and can be coupled with SFE to produce a customized end product or fraction. Whereas the traditional first step in Figure 2 was usually extraction-based (i.e. the removal of caffeine from coffee to produce a decaffeinated product, or isolation of a hops extract for flavoring), today's processes can utilize a supercritical fluid fractionation (SFF) process, either initially or coupled with SFE, to produce value-enhanced products. Recently, the recognition that critical fluids offer a unique processing media in which to conduct reactions (6), opens but yet another option to the food and agriproduct processor.

In this brief review we wish to cite from our own research and others, the changing nature of the field of critical fluid processing, and its implications for the food industry. The applications cited have not as yet reached actual production practice, but they illustrate some of the possibilities for new processing options, and ultimately new products that can be produced in an environmentally benign way, with no objectable solvent residuals, and potentially high consumer appeal.

Fractionation techniques

Prior to the mid-1980's, critical fluid-processed products were largely derived using SFE; either by selecting a given fluid density that would yield the desired product, altering the extraction fluid density as a function of processing time, or in some cases selectively decreasing the pressure after the extraction stage to achieve the desired extract. Useful separations have been attained using the above techniques (7), but largely between compounds differing significant in their physicochemical properties, e.g., molecular weight, vapour pressure, polarity.

Recently, fractionation processes utilizing critical fluid media has been improved by combining principles utilized in supercritical extraction with other separation techniques. These improved methods often make use of fractionating columns or chromatography to yield improvements in the resolution of molecular species. The fractionating column or tower approach is somewhat analogous to operating a distillation column, but there are differences when using critical fluid media. For an understanding of the fundamentals involved in using this technique, one should consult the primer by Clifford (8).

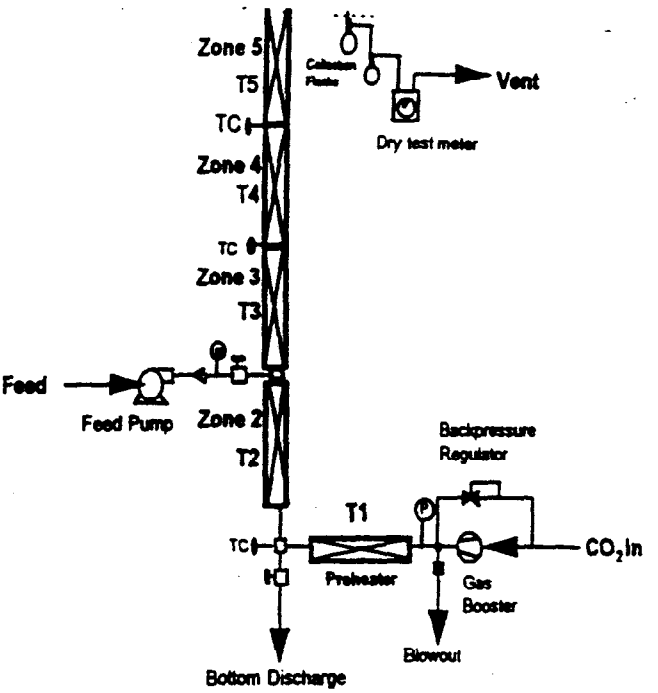

Figure 3 - Schematic of a thermal gradient packed column fractionation system.

Figure 3 illustrates the components and principles involved in fractionating using a very simple column. In this case, SC-CO_2 is brought to the desired pressure and then directed to flow upward inside the fractionating column, which usually contains a packing to encourage contact between the SC-CO_2 and the components being separated. The components to be separated are injected with a pump into the flowing SC-CO_2, prior to its entry into the column. This fluid-solute mixture than enters the first heated zone in the vertical fractionating column and the separation process is initiated. The SC-CO_2 - solute mixture than ascends the column, usually encountering zones of increasing temperature, which facilitate the separation of solutes based on their relative solubilities in SC-CO_2 and their respective vapour pressures. In effect, the column is operating under a density gradient since the fluid is kept isobaric.

The described fractionating column can be operated in either the batch or semi-continuous mode with co-current flow of the solute and supercritical fluid streams. Using this approach, we and others have demonstrated the enrichment of monoglycerides from a concentration of 45 weight % in a mixed glyceride starting material, to over 95 weight % content in the extract taken off the top of the column (9). Similarly, in recent studies designed to attain an enriched nutraceutical- rich extract (10) , we have removed contaminant free fatty acids from vegetable oil deodorizer distillates and enriched the free sterols and steryl ester content of the raffinate as presented in Table 1. Note that for both rice bran and soybean oils, the starting material (the feed into the column) contained between 32-38 weight % free fatty acid and 13-18 weight % sterols,

Table 1 - Raffinate compositions (weight %) from fractionation tower separation of vegetable oil deodorizer distillates (DD) using SC-CO2.

Component	Rice Bran DD	Soybean DD
Free fatty acids (FFA)	5	8
Sterols	20	31
Steryl esters (StE)	7	3
Triglycerides (TG)	38	30

while the raffinate collected after the fractionation, had a free fatty acid content of between 5-8 weight % and sterol-steryl ester enrichments of 27-34 weight %. These results could undoubtably be improved upon by employing an even longer fractionating column or by preferentially operating in the countercurrent mode, that other investigators have employed in Germany (11) and Italy (12).

Another SFF option is to employ chromatography in the preparative or production mode, in its own right, or coupled with a preliminary SFE enrichment stage. Our research group at NCAUR has utilized the latter approach several times to achieve extracts with target compounds enriched at levels that were not possible by using SFE alone. Figure 4 illustrates the principle involved in this form of fractionation when using SC-CO_2, both with and without an organic co-solvent, in either the SFE or supercritical fluid chromatography (SFC) stages.

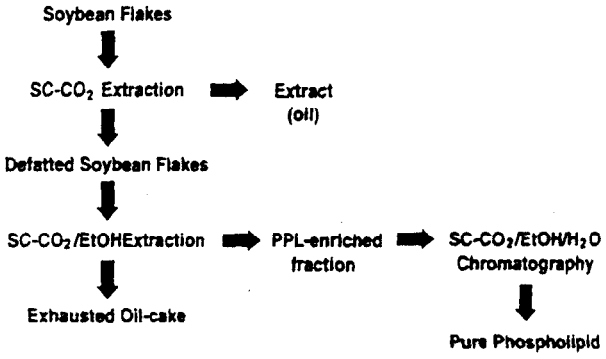

Figure 4 - Phospholipid enrichment/fractionation by combined SFE and SFC.

Here a preliminary optimized SFE step is utilized to achieve the best possible enrichment of solutes prior to introduction of the extract to the chromatographic column. Then, using inexpensive commodity sorbents whenever possible, the extract from the extraction stage is diverted onto the top of the chromatographic column, where it is further fractionated using either neat SC-CO_2, or SC-CO_2 with ethanol and water as eluents.

Using this approach, we have been able to enrich moieties such as tocopherols, phospholipids, or steryl esters from vegetable oils, seeds, and by-products of the milling or vegetable oil refining processes. Figure 4 is for the specific case for the separation, enrichment, and fractionation of phospholipids (PLs) from vegetable oil or seeds (13). Here soybean flakes are initially extracted with SC-CO_2 to remove the oil from flakes, followed by extraction of the PLs from the deoiled flakes with a SC-CO_2/ethanol mixture. The second extraction step produces an extract enriched in PLs since PLs are not appreciably soluble in neat SC-CO_2, but can be selectively removed from the flake matrix with the aid of ethanol as a co-solvent.

As shown in Table 2, the second SFE using SC-CO_2/ethanol produces an extract containing a total 43.7% by weight of PLs. This is a considerable enrichment relative to the concentration of the PLs in the starting oil or seed matrix. Further PL enrichment is facilitated as noted above, by transferring this extract enriched in PLs to an alumina preparative SFC column, where SC-CO_2 modified with a 5-30 vol.%, 9:1/ethanol:water eluent is used to elute and fractionate the PLs. In the case of the SFC enrichment step, eluent fractions can be collected as a function of time and their PL content quantitated. As indicated by the data given in Table 2, collection of discrete fractions during the SFC process can produce purities in excess of 75% for the individual PLs, phosphatidylcholine and phosphatidylethanolamine. It should be noted in the described process, that in the SFC steps, that only GRAS (Generally Regarded As Safe) solvents are being used for the enrichment process.

Recently, a similar SFE/SFC process has been used to isolate sterols and phytosterol esters from agricultural by-products such as corn bran and fibre (14). For example, by using both SFE and SFC, it has been possible to isolate a fraction containing up to 53% by weight of a cholesterol-lowering agent, ferulate phytosterol esters (FPE) from corn fibre oil. Enrichment of these types of nutraceutically-active compounds with 'natural' agents such as CO_2, ethanol, or water, creates an attractive additive package for implementing unique functional food composition; as well as demonstrating the potential of SFF.

Table 2. Percentage amounts of PLs in extracts derived from SFE and SFC processing stages*.

Phospholipid Type	SFE Stage	SFC Stage
Phosphatidylethanolamine	16.1	74.9
Phosphatidylinositol	9.2	20.8
Phosphatidic Acid	2.8	55.8
Phosphatidylcholine	15.6	76.8

* Relative to other eluting constituents (oil and unidentified peaks)

Table 3 - Effect of water content of glycerol (weight %) on the product distribution from soybean oil glycerolysis using SC-CO_2[a]

Components	0.7% H_2O[b]	4.2% H_2O[b]
Free fatty acid	N.D.[c]	1
Monoglycerides	84.0	67.0
Diglycerides	15.4	28.9
Triglycerides	0.6	3.1

[a] Conditions: Corn oil and glycerol flows 10 microliter/min, 70°C, 27.6MPa. [b] Product distributions in weight %.
[c] N.D. = non detected.

Selective reaction chemistry in critical fluids

There is a historical precedent for conducting specific reactions under supercritical conditions, such as the polymerization of ethylene or isomerization of olefins. In these reactions, it is often just the application of pressure as a thermodynamic variable which permits the reaction equilibria to be shifted, yielding the desired end products. However since the early 1990's, there has been an increasing awareness that by conducting reactions in critical fluid media, one can control not only the equilibrium position of a reaction, but the product distribution and end properties (i.e. colour or morphology).

Two types of reactions which we have studied that have implications for the food industry are enzymatic-catalyzed and hydrogenation reactions. The coupling of enzyme catalysis with such critical fluids as CO_2 is particularly attractive, since both are 'natural' agents which allow food components to be manufactured without introducing non-permissible solvent or catalyst residues into the final product. In particular, we and others have demonstrated that lipases can be used in the presence of SC-CO_2 to conduct the following reactions of importance to the food industry (15): hydrolysis, esterifications, transesterifications, glycerolysis, and randomization of fats, among others.

The above transformations can be conducted in the presence of a lipase, but particular attention must be paid to the temperature, pressure, and presence of water in the supercritical fluid system. Reactions of this type can be conducted in either batch or flow systems, the latter being attractive for the continual production of the desired product. Of particular note is the use of Novozym 435, a lipase derived from *C. antarctica*, as a catalyst supported on a polyacrylate resin. This enzyme has proven particularly effective catalyst in the presence of SC-CO_2, although the need for more pressure- and temperature-resistant lipases would be a welcomed addition for use with critical fluid media.

An excellent example of the versatility offered using enzymatic catalysis using flowing SC-CO_2 is the glycerolysis of soybean oil with Novozym 435. The resultant glyceride mixtures find a wide range of application in food and related industries as emulsifiers and surface active agents. The described process has been patented by us, but details are available in a related publication (16). To initiate the glycerolysis in SC-CO_2, the vegetable oil is fed into the SC-CO_2 by a pump, where it is dissolved in the compressed fluid along with an excess of glycerol containing a small quantity of water. This mixture is then passed over the Novozym 435 supported enzyme bed in the reactor vessel, where total or partial glycerolysis takes place to yield glyceride mixtures.

The overall yield and composition of the resultant glyceride mixture is dependent on a number of adjustable variables, such as flow rate of reactants or water content of the glycerol. As shown in Table 3, one can change the glyceride product distribution obtained from the reaction, by varying the water content of the reactants. Table 3 indicates that by using a relatively low water content, a glyceride mixture having a high monoglyceride content (84%) can be obtained, however by increasing the water content in the glycerol reactant solution to 4.2% the monoglyceride content in the final product can be reduced to 67% by weight. The resultant glyceride mixtures also tend to be much paler in colour when using the SC-CO_2-based synthesis method, a fact confirmed in a related study involving glyceride synthesis in the batch mode (17).

Hydrogenation in supercritical fluid media has attracted considerable interest, based to a large extent, on the superior mass transfer characteristics achieved when using hydrogen mixed with SC-CO_2. Due to several factors, contact of the dissolved hydrogen with catalyst and substrates is facilitated in both flow and batch reactor systems using a H_2/SC-CO_2 mixture. Recent studies by Harrod and colleagues (18) and others (19) have demonstrated that increased hydrogenation rates are possible when synthesizing fatty alcohols from fatty acid methyl ester substrates, as well as for the hydrogenation of vegetable oils.

Recently we have conducted experiments in batch reactor systems at slightly elevated pressure (up to 2000 psi) in which hydrogen is admixed with carbon dioxide in a vessel containing a Ni commercial catalyst and soybean oil. Using mixtures of H_2 and CO_2 at equal pressures and temperatures between 120-140°C, a variety of hydrogenated oils can be produced that have varying iodine values (IV), % trans fatty acid and solid fat indices (SFI). It is particularly interesting to note that the SFI are relatively invariant with respect to temperature and can be adjusted by varying the ratio of H_2 to CO_2 pressure.

It is possible using this technology to produce equivalent or better hydrogenated oil basestocks for use in margarine or shortenings, relative to those obtained using conventional technology. As shown in Table 4 (overleaf) oils with lower trans fatty acids are possible using the above technique, relative to an equivalent commercial product of like IV value, produced by conventional hydrogenation. Thus potential useful margarine and shortening basestocks can be produced by this method having the dropping point (DP) characteristics stated in Table 4.

Table 4. Properties of potential vegetable oil-derived margarine/shortening using hydrogen/ carbon dioxide mixtures during hydrogenation.

Oil Property	Margarine basestock (D.P. 32-39°C)		Shortening basestock (D.P. 45-52°C)	
	Conventional	Experimental	Conventional	Experimental
% Stearic acid	6-9	7-11	11-13	13-24
% Trans	11-30	1-3	15-20	3-8
IV value	90-110	108-114	85-90	88-102

Subcritical water as a processing medium for food production

The success in applying both SC-CO_2 and liquid CO_2 as benign solvents for the extraction of foodstuffs has catalyzed the search for other alternative extraction media that are acceptable in food processing. During the past five years, subcritical water (remember Figure 1), that is H_2O held in its hot pressurized liquid state, has become of interest, as reported in the research of Hawthorne and associates (20). Initially, application of this medium to biological or organic materials was somewhat tentative, due to concerns of reactivity and subsequent degradation of solutes under elevated temperatures. (Note - this is not the technique of supercritical water oxidation which is usually conducted above the critical temperature of water, 375°C, for the expressed purpose of degrading organic wastes, etc.). However, recently this medium under a restricted temperature range, has been applied successfully for the extraction of food materials and natural products by Clifford at the University of Leeds in the United Kingdom (21).

The solvent power of subcritical water is largely varied by changing temperature and applying enough external pressure to eliminate vaporizing the water when used above its normal boiling point. Hence, by varying the temperature of water over a range of 25 - 250°C, one can change its effective dielectric constant from 78 to under 30, thereby attaining solvent polarities normally associated with polar organic solvents. Thus, in theory, subcritical water is a perfect compliment to SC-CO_2 for processing foods and thereby avoiding the use of organic solvents. The potential reactivity of solutes as noted above, suggests that temperatures between 100-175°C are optimal for most food and natural product applications; but as the author and his colleagues have shown, hydrolysis (22) under subcritical water conditions is a feasible transformation process. Further, the possibility of creating flavour precursors under conditions akin to those obtained in aqueous-based cooking processes suggests that reactivity under subcritical water extraction conditions may not be entirely negative in effect.

As opposed to some of the more optimal conditions for SC-CO_2 extraction, subcritical water extraction does not require any prior drying of the substrate before the extraction step, and can be optimized to avoid the extraction of waxes and other lipid material from plant matter. Clifford *et al.* (23) have have nicely demonstrated the utility of subcritical water as an extraction medium for the removal of oxygenated compounds from rosemary, clove buds, and the deterpenation of lemon oil. As shown in Table 5 (below), the yields of various components in milligrams from a four gram sample of rosemary, are somewhat better using pressurized hot water extraction at 150°C, than by applying steam distillation. Other factors, such as the selectivity for oxygenated solutes over monoterpenes and the higher heat recovery versus steam distillation, make subcritical H_2O an attractive processing technique. Recently, countercurrent deterpenation of citrus oils has been demonstrated on a continous basis using subcritical water at the University of Leeds, and is being currently scaled up at Express Separations Ltd. in Yorkshire.

Table 5. Comparison of yields from rosemary extraction by steam distillation and subcritical water extraction at 150°C*.

Compound	Steam distillation	Subcritical water extraction
alpha-pinene	10.9	4.0
Camphene	2.0	1.5
Limonene	1.4	1.3
1,8 - cineole	2.7	5.3
Camphor	3.4	6.4
Borneol	2.1	3.5
Verbenone	5.0	6.3
Isobornyl acetate	1.0	1.5
Unidentified sesquiterpene	0.7	0.01
Total	50.7	54.2

* Yields in mg per 4 grams of sample

Concluding remarks

In this brief review we have attempted to document some of the current trends in critical fluid technology as applied to food processing. The use of critical fluid media from various regions of the phase diagram (Figure 1) is becoming an increasing trend as well as the use of critical fluids in conjunction with a liquid solvent that has GRAS approval. Although simplistic, the author has reason to believe that many food component extraction and separation problems could be solved by the combination of carbon dioxide, ethanol, and water; as an 'all-natural' trio of solvents. Supercritical processing is also beginning to merge with high pressure hydrostatic processing (24) and the prophylactic benefits of a residual CO_2 atmosphere in SFE-products has been documented. Hopefully this review has demonstrated that critical fluid technology as applied in the food and agricultural processing industry continues to be a dynamic area of research and development, but has undergone considerable change since the 1970's when it was first applied in food processing.

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Foot note

This review expands on a presentaion by Dr King at the meeting, *'Cutting-Edge Advances in Food Processing Technology'* sponsored by the SCI Food Commodities & Ingredients Group, IFST and IFTBS, held on 22 September 1999 at the SCI, Belgrave Square, London.